

# Tpt1 Cas9-KO Strategy

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Reviewer: Zihe Cui

**Design Date: 2021-1-11** 

## **Project Overview**



**Project Name** 

Tpt1

**Project type** 

Cas9-KO

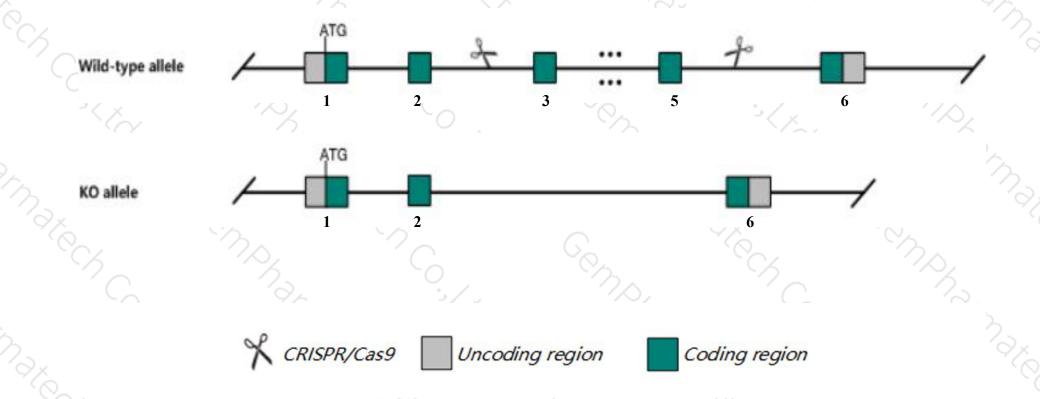
Strain background

C57BL/6JGpt

## **Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the *Tpt1* gene. The schematic diagram is as follows:



### **Technical routes**



- The *Tpt1* gene has 8 transcripts. According to the structure of *Tpt1* gene, exon3-exon5 of *Tpt1-201*(ENSMUST00000110894.8) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Tpt1* gene. The brief process is as follows: CRISPR/Cas9 system were microinjected into the fertilized eggs of C57BL/6JGpt mice. Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

### **Notice**



- > According to the existing MGI data, mice homozygous for a knock-out allele die prior to embryo turning due to defects in embryogenesis.
- > The Intron2 and Intron5 are only 512bp and 738bp,loxp insertion may affect mRNA splicing.
- The KO region deletes most of the coding sequence, but does not result in frameshift.
- The KO region may contains the regulation functional region of the *Gm4285* gene and *Snora31* gene. Knockout the region may affect the function of *Gm4285* gene and *Snora31* gene.
- The *Tpt1* gene is located on the Chr14. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- This strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risk of the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

## Gene information (NCBI)



#### Tpt1 tumor protein, translationally-controlled 1 [ Mus musculus (house mouse) ]

Gene ID: 22070, updated on 22-Nov-2020



☆ ?

Official Symbol Tpt1 provided by MGI

Official Full Name tumor protein, translationally-controlled 1 provided by MGI

Primary source MGI:MGI:104890

See related Ensembl: ENSMUSG00000060126

Gene type protein coding
RefSeq status VALIDATED
Organism Mus musculus

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae;

Murinae; Mus; Mus

Also known as Tr; fo; TCT; Trt; p21; p23; TCTP

Expression Ubiquitous expression in ovary adult (RPKM 1476.1), bladder adult (RPKM 983.1) and 28 other tissues See more

Orthologs human all

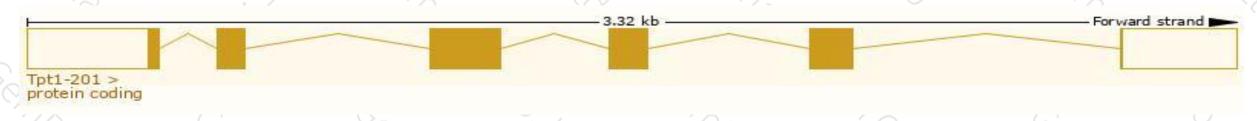
## Transcript information (Ensembl)



The gene has 8 transcripts, all transcripts are shown below:

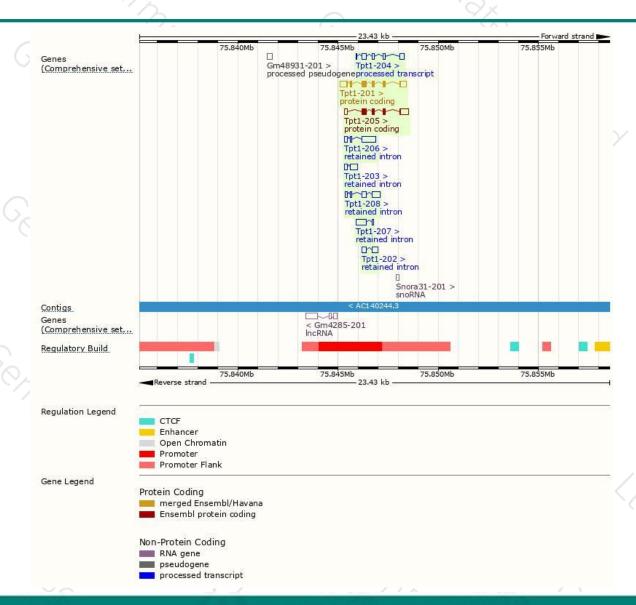
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tpt1-201	ENSMUST00000110894.8	1169	<u>172aa</u>	Protein coding	CCDS36980	P63028	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Tpt1-205	ENSMUST00000142061.2	996	<u>138aa</u>	Protein coding	-	D3YU75	TSL:5 GENCODE basic
Tpt1-204	ENSMUST00000134040.1	661	No protein	Processed transcript	=	- 21	TSL:3
Tpt1-206	ENSMUST00000150047.7	890	No protein	Retained intron	-	2:	TSL:1
Tpt1-208	ENSMUST00000154533.7	762	No protein	Retained intron	ā.	-	TSL:2
Tpt1-207	ENSMUST00000151751.1	559	No protein	Retained intron	-	-0	TSL:2
Tpt1-202	ENSMUST00000125146.1	503	No protein	Retained intron	=	-	TSL:2
Tpt1-203	ENSMUST00000128889.1	478	No protein	Retained intron	-	2:	TSL:2

The strategy is based on the design of *Tpt1-201* transcript, the transcription is shown below:



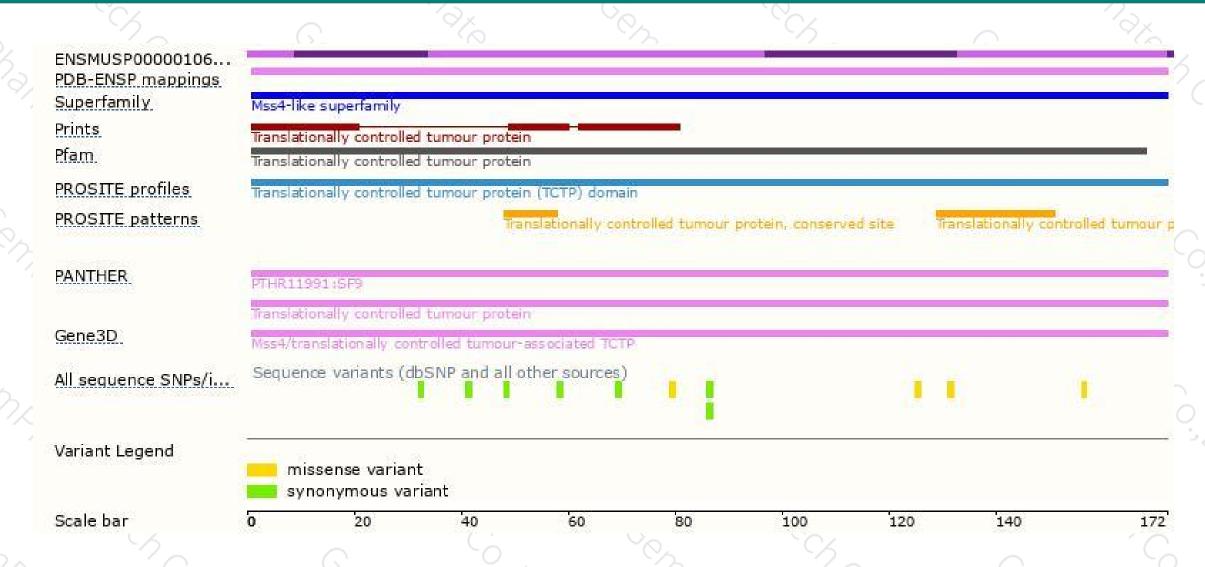
### Genomic location distribution





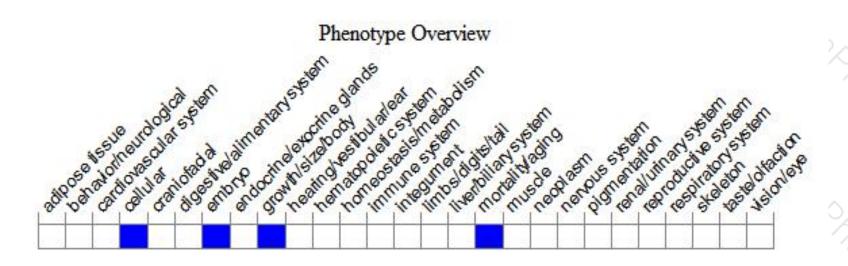
### Protein domain





## Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data, mice homozygous for a knock-out allele die prior to embryo turning due to defects in embryogenesis.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





